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Karyotype and colchicine diversity in different morphotypes of *Gloriosa superba* L. of West Bengal

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Abstract: *Gloriosa superba* L. is medicinally important plant species containing as well as having some ornamentals and horticulture values. The plant was under threatened category due to its imprudent harvesting from wild as it extensively used by medicinal industries for its colchicine content. It is available throughout West Bengal, India with high variability in morphological and colchicine content. The present investigation on *Gloriosa superba* L. deals with detailed comparative study of somatic chromosome and phytochemical analyses of six morphotypes of West Bengal. In view of this, cytological races have been already observed in this species and a detailed karyotype analysis which were found to be diploid in all the morphotypes. The present investigation revealed that the 2n= 22 chromosomes are in deep seated of this species. The karyomorphological variations in different morphotypes may be due to the minute structural changes of chromosomes.

Keywords: *Gloriosa superba*, Different morphotypes, Karyotype, Somatic chromosome, Colchicine analysis.

1. Introduction

Gloriosa superba L. is the member of the family Colchicaceae (according APG system). This species is native in Africa; however, it occurs throughout the tropical climatic zones ascending to 6, 000 ft. on the hills (Jana and Shekhwat, 2011). In India, this species is common in Mysore

state and other parts of South India (Maroyi and Maesen, 2011; Vijoyavalli and Mathew, 1990). In West Bengal, too *G. superba* is widely distributed extending from South 24 Parganas to the hilly region Lava (Darjeeling) at 2134m. altitude. This species is perennial climbing herb and it is economically important for its ornamental and its diverse medicinal values (Jana and Shekhawat, 2011). Due to over abuse for commercial medicinal applications, this plant is facing local loss in some areas of India, Srilanka, Bangladesh and South Africa. This species is now declared as endanger plant by IUCN (Ade and Rai, 2009). The medicinal values of *G. superba* L. have been reviewed. Different morphological part of *G. superba* L. are used in leprosy, parasitical affections of skin, piles, colic, in snake- bites and scorpion stings (Jana and Shekhwat, 2011). More significantly, alternative source of colchicine other than *Colchicum autumnale*, *G. superba* L. is recognized as the source of colchicine (Ghosh *et al*., 2002). It was reported that *Colchicum luteum* Baker was the only source of colchicine (Gupta and Raina, 2001). But presently *G. superba* is considered as alternative source of colchicine (Ghosh *et al*., 2002). Colchicine is used as a powerful antimitotic agent in plant breeding programme. Other than, the antimitotic property, the alkaloid colchicine is widely used for different plant based drug (Jana and Shekhwat, 2011). The tubers of this herb are the main source of colchicine of Indian's export market and earn foreign exchange value (Kavina *et al*, 2011). The commercial products, which are available in markets, are La Medicca (India) Private Limited: Essential Oil, Ayurvedic Kalahari (Model No. - COPLON09) etc.

All cytological studies of *G. superba* L showed 2n= 22 chromosomes, Fedorov, 1969 and Goldblatt, 1984. However, Nwofia *et. al*., 2001; chromosome number of South African species of this species reported 2n= 56 chromosomes with symmetrical karyotype containing 44 median and 12 submedian chromosomes. Ghosh *et al*., 2008, reported tetraploid species of *G.superba* L. with 2n= 4n= 44 chromosomes. Sharma and Sharma, 1980; Vijoyavalli and Mathew, 1990, showed symmetrical karyotype. All this published reports of karyotype and cytological of *G. superba* L. is the positive indication of biological diversity of this species.

The present investigation carried out detail karyotypes of six morphotypes of *G. superba*, five of which from Southern parts of West Bengal at sea level altitude in different edaphic zones and one from Northern part of West Bengal, Lava (Darjeeling district) at 2134 meters altitude. The objective of this study to produce chromosomal database of different morphotypes of West

Bengal, India and to interpret intraspecific relationship based on karyomorphology of this species along with colchicine contents to find out co-relationship among them. In this connection the chromosomal diversity of *G. superba* L in our study also want to highlight the karyotypical diversity of six morphotypes and their colchicine content which can create a base line data for future important programme.

2. Material and Methods

Plant material of *Gloriosa superba L.*

G. superba L is a climbing herb with leafy system springing from a naked tuberous root stock. The leaves are alternate, sessile, lanceolate. The leaf tips are trendily like which help for climbing. The flowers are large, showy, solitary at ends of branches, greenish at first then yellow, passing through orange and scarlet to crimson (Jana and Shekhawat, 2011). Perianth lobes six in number petaloid bent backwards. Stamen six radiating, the style bent almost 90º at the point of attachment to the ovary. Fruits are oblong, ellipsoid capsule. Seeds are numerous and rounded (Gupta and Raina, 2001).

Life forms of six morphotypes of this species were collected from different regions of West Bengal, India. Five of them were from (i) Rahara, North 24 Parganas (morphotype -I); (ii) Narendrapur, South 24 Parganas (morphotype -II); (iii) Sargachi, Murshidabad (morphotype -III); (iv) Kalyani, Nadia (morphotype -IV); (v) Benapur, Medinipur (morphotype -V) at sea level altitude and one from (vi) Lava, Darjeeling (morphotype -VI) of 2134-meter altitude (Table-1). All this morphotypes show variation in external morphology (Table- 2).

Methods for chromosome study

Roots meristems germinating from the tuber of each morphotypes were pretreated in 0.5% colchicine followed by fixation in 3:1 aceto- ethanol for overnight at 4ºC, kept in 45% acetic acid for 20 minutes, hydrolyzed in 1N HCl for10 minutes at 60ºC and stained with 2% aceto- orcein. After, several trails with methods of pretreatment, fixation and hydrolysis were performed before reporting the final method. Ten metaphase plates per morphotypes were thoroughly examined under the microscope. Well- spread metaphase plates were documented with the help of microscope (Leica) with camera (Leica DFC295). At the same time, Camera Lucida drawing of

metaphase plates was drawn by using 100x × 15x under the microscope of 2000 magnification. Magnification of the microscope was measure by Camera Lucida drawing of stage micrometer using 100x × 15x. Lengths of short and long arms of each chromosome were calculated. These values were then used to calculated total chromatin length (TLC) and centromeric index (CI) of each morphotypes. Arrangement chromosomes in grouping of each karyotype were made on the basic of centromeric index, and ordering them by decreasing length with in each group. The mean centromeric index value (TF%) of each morphotypes was calculated by using Huziwara,1962 formula:

TF% = (total sum of short arm length/ total sum of chromosome length) × 100. At the same time mean chromatin length and total arm ratio - (total long arm length/ total short arm length) calculated by using Levan *et al*., 1964 formula (Table- 4), in that arm ratio 1= M (metacentric), arm ratio 1.7 = m (median region), arm ratio 3.0= sm (sub-metacentric), arm ratio 3.0= st (sub terminal and arm ratio 7.0= t (acrocentric terminal).

Extraction of Colchicine from tuber

Tubers of *G. superba* collected from six different morphotypes of West Bengal were quantitatively and qualitatively assessed for the presence of colchicine. For this purpose, ten randomly chosen plants of each morphotypes were taken. Colchicine was extracted using a standard method and analyzed (Hayashi *et al*., 1988, Ghosh *et al*., 2002). For extraction, tubers were washed thoroughly by running tap water then sliced, dried and powdered by electric grinder. Approximately 25 gm of sample were taken into a thimble and placed in a Soxhlet apparatus, were set up with methanol tuber (B. P= 65°C) solvent. The extraction was carried out for 8 hours to ensure the complete extraction of colchicine from the dried tubers of *G. superba*. After completion of extraction the dark brown extract was then cooled, filtered (Watman, Grade No.-1), concentrated using rotary evaporator, and finally by vacuum suction to get a crude dried extract which was yellowish black in color. Weighed accurately 8 gm sample and dissolved in 80 ml methanol and finally made up to1mg/ml, filtered the sample through 0.2 µm membrane filter before analysis (Lot no. T70340). The methanolic extraction was used for qualitative and quantitative analysis.

Qualitative and quantitative analysis of colchicine

For qualitative analysis of colchicine in the samples of tuber extract of *G. superba*, the methanolic extract was concentrated and subjected to TLC. The analysis of extracted colchicine was done with High performance thin layer chromatographic (HPTLC) technique. TLC plates (20×10 cm) coated with layers of silica gel 60 F254 (Merck, India, Cat No. 1.05554.007, Batch no. HX245754) was used. These spots were applied at 20 mm distance and allow drying. Chloroform: Acetone: Diethylamine (5:4:1) V/V was taken as mobile phase in development chamber and allowed to saturate for 20 min. One major band which was detected visually and under UV 353 nm in the plate of colchicine extract. The distance travelled by Colchicine was 8 cm while solvent was up to 8 cm. The amount of Colchicine was measured with calibration curve of standard Colchicine.

The major compounds present in the crude mixture of tubers of *G. superba* L., extract were also analyzed and quantified by High performance liquid chromatography (Waters 600/2998), briefly, a Waters Nova – Pak ® C18 (3.9 mm_300 mm, 4 uM particle). As a mobile phase water (A): acetonitrile (B) with 0.1% acetic acid was taken. A 20 µL crude mixture dissolved in methanol was injected and identified with standard being detected at 254 nm wavelength, using uv detector. The flow rate was kept at 1 ml/min. The gradient was set as starting at 92% solvent A and ramping to 75% solvent A in 5 min, holding at 75% solvent A for 2 min, ramping again to 100% solvent B in 10th min and finally backing to 92% solvent A in next 9 min. The concentration of compound colchicine was calculated from the area of the standard injected at the same parameters.

Identification of colchicine was done comparing the retention time of the sample with that of authentic colchicine (Himedia, Cat no.PCT1302-1G). All samples were extracted and analyzed in triplicate.

3. Results

Morphological variations in different morphotypes

Variations in morphology were study in the plants of six collected morphotypes. The plants of morphotype -VI were distinguishable different from the plants of other five morphotypes and

morphotype- I was also distinguishable from other morphotypes. The plants of morphotypes -II, III, IV and V were morphologically related. Details morphological characters and measurements (leaf length, leaf width, intermodal length, tubers size, diameter of tuber, flower color and length of petals) are summarized in Table-2. Measurements of 1-year-old tubers were taken for analysis, only mature leaves and mature flowers were measured to determine leaf length, leaf width, internodal length and flower color, length of petals for this study. Plants of morphotype-VI were smallest than other morphotypes but tubers size (12.36 ± 0.2) and diameter of tubers (2.12 ± 0.05) were also largest. In morphotypes- III, tubers size (7.6 ± 0.15) and diameter of tubers (1.4 ± 0.04) were smallest among the all morphotypes but plants were larger than morphotypes- VI. Leaf length (10.0 ± 0.1) of morphotype-II was largest than other morphotypes and leaf length (5.2 ± 0.4) of morphotypes- V was smallest among the all morphotypes but leaf width (4.5 ± 0.1) and internodal length (8.76 ± 0.2) was largest than other morphotypes. Leaf width (2.0 ± 0.4) of morphotype- VI was smallest among the other five morphotypes. In morphotypes- I, III and V, leaf length and leaf width were nearly similar size. Internodal length (5.0 ± 0.15) of morphotype- V was smallest among the all morphotypes. Flower color of morphotype-V was also distinguishable and noticeable from other morphotypes which were upper red and lower greenish in color (Fig. - F) and petals length was 4.8 ± 0.2. Petals length (10.1 ± 0.1) of morphotypes- I was largest among the all morphotypes and flower color was upper pinkish, lower white in color (Fig. - A). In morphotypes- II, flower color was off yellow with arid patch along the central region (Fig. - B) and petals length (3.8 ±0.3) was smallest then other morphotypes. The details morphology of the six different morphotypes was of collections are shown in Figure- 1, 2, 3.

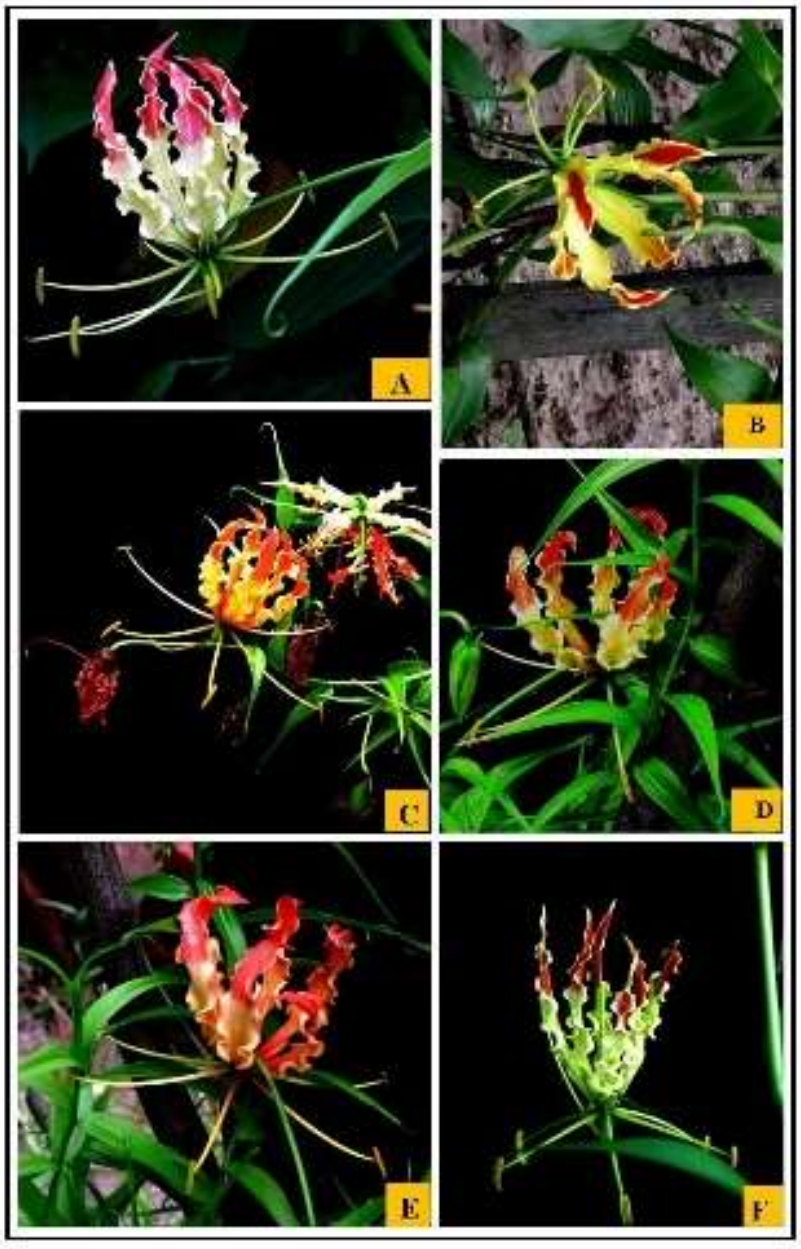


Fig. 1

Fig. 2

Fig. 3

1. **Fig. 1.** (A- F); Showing flowers the plants of *Gloriosa superba* L. collected from A– Rahara, B– Narendrapur , C– Sargachi, D– Kalyani, E– Benapur, F– Lava.

2. **Fig. 2** (i– vi), Showing rizomes of the plants of *Gloriosa superba* L. collected from i– Rahara , ii– Naredrapur , iii– Sargachi, iv– Kalyani, v– Benapur, vi– Lava.

3. **Fig. 3** (i– v), Showing rhizomes of the plants of *Gloriosa superba* L. collected from i– Rahara , ii– Naredrapur , iii– Sargachi, iv– Kalyani, v– Benapur, vi– Lava.

Table-1: Geographical details of the six Morphotypes of West Bengal of *Gloriosa superba* L.

Sl. No.	Morphotype	Location	Geographical position	Altitude	Soil types	Temperature during harvesting season
1.	I	Rahara (North 24 Parganas, West Bengal, India)	Latitude- 22.72°N Longitude- 88.38°E	16 meters above the sea level	Loamy soil	35°C
2.	II	Narendrapur (South 24 Parganas, West Bengal, India)	Latitude- 22°11'6"N Longitude- 88°20"E	4 meters above the sea level	Sandy Loam	32°C
3.	III	Sargachi (Murshidabad, West Bengal, India)	Latitude- 19°18'N Longitude- 84°51' E	19 meters above the sea level	Slightly acidic, mainly gengetic alluvial soil	27°C
4.	IV	Kalyani (Nadia, West Bengal, India)	Latitude- 22°53'N Longitude- 88°9' E	7.8 meters above the sea level	Gengetic alluvium, light texture	30°C
5.	V	Benapur (Medinipur, West Bengal, India)	Latitude- 22.4333°N Longitude- 87.3333° E	23 meters above the sea level	Sandy Loam	32°C
6.	VI	Lava (Darjeeling, West Bengal, India)	Latitude- 27.0500°N Longitude- 88.2667°E	2134 meters above the sea level	Predominantly reddish, yellowish in color mainly mixed sandy loam and brown forest soil.	22°C

Table-2: Gross morphological analysis of different Morphotypes of *Gloriosa superba* L. Values represent means ± standard error of three experiments with 10 replicates.

Leaf length (cm.)	7.6 ± 0.2	10.0 ± 0.1	7.4 ± 0.3	8.8 ± 0.1	5.2 ± 0.4	6.6 ± 0.3
Leaf width (cm.)	4.4+ 0.1	4.3 + 0.2	4.4 + 0.5	4.4 + 0.1	4.5 + 0.1	2.0 + 0.4
Internodal length (cm.)	5.94 ± 0.13	6.32 ± 0.19	5.0 ± 0.15	5.08 ± 0.07	8.76 ± 0.2	5.88 ± 0.1
Tuber size (cm.)	10.21 ± 0.13	10.34 ± 0.14	7.7 ± 0.15	7.6 ± 0.16	8.82 ± 0.23	12.36 ± 0.2
Diameter of Tuber (cm.)	1.51 ± 0.02	1.62 ± 0.03	1.4 ± 0.04	1.34 ± 0.06	1.7 ± 0.05	2.12 ± 0.05
Flower color	Upper pinkish and lower	Off yellow in color with arid	Lower light yellowish and	Lower whitish-yellow and upper	Lower creamy and upper red in	Upper red and lower greenish in color
Length of petals	10.1 ± 0.1	3.8 ± 0.3	5.0 ± 0.2	4.9 ± 0.4	9.5 ± 0.2	4.8 ± 0.2

(Morphotypes details: I- Rahara, II- Narendrapur, III- Sargaachi, IV- Kalyani, V- Benapur, VI- Lava)

Cytological variations in different morphotypes

Karyomorphological studies of the six morphotypes of *G. superba* L. were made (Table-3). Somatic chromosome of all were found 2n= 22 (Figure- 4, 5). In this study, the chromosomes of the *G. superba* L. morphotypes were grouped into the following four types:

Type A: Relatively long to short chromosomes (length- 4- 9.5 µm) with primary and secondary constrictions dividing the chromosome into three more or less equal arms.

Type B: Relatively long chromosomes (length- 5- 7.5 µm) with primary and secondary constrictions, the median arm is small.

Type C: Relatively long to short chromosomes (length- 4- 11.5 µm) with median to nearly median primary constriction of chromosomes of this type is the longest among the total chromosome complement.

Type D: Relatively long to short chromosomes (length- 3.5- 5.5 µm) with submedian to nearly submedian primary constrictions.

Descriptions of the metaphase chromosomes of individual morphotypes are given bellow:

Morphotype-I: The chromosome number of this type was 2n= 22 with the Karyotype formula 2A + 2B + 8C + 10D (Table- 3). The ranges of the lengths of chromosomes were 4µm - 10µm. One pair of chromosome was relatively long to short chromosomes (6.5 µm) with primary and secondary constrictions dividing the chromosome into three more or less equal arms and one pair of chromosome was relatively long chromosomes (6µm) with primary and secondary constrictions, the median arm is small. Four pairs of chromosomes showing relatively long to short chromosomes with median to nearly median primary constriction of chromosomes (length- 4- 10µm) is the longest among the total chromosome complement and five pairs of chromosomes showing relatively long to short chromosomes (length- 4.5- 6 µm) with submedian to nearly submedian primary constrictions. The percentage of metacentric chromosome except secondary constriction was 36.4% and the percentage of sub- metacentric chromosome except secondary constriction was 45.5% of this morphotypes. The total centromeric index was 38.18% and the total chromatin length was 62.5± 0.12 µm. The arm ratio of the total chromosomes of this

morphotypes was 2.46. One pair of interstitial secondary constrictions was in this morphotypes. One pair of chromosome (length- 10µm), which was longest among the all chromosomes in this morphotypes.

Morphotype-II: The chromosome number of this type was 2n= 22 with the Karyotype formula 2B + 14C + 6D (Table- 3). The ranges of the lengths of chromosomes were 5µm – 11.5µm. One pair of chromosome was relatively long chromosomes (6µm) with primary and secondary constrictions, the median arm is small. Seven pairs of chromosomes showing relatively long to short chromosomes with median to nearly median primary constriction of chromosomes (length- 5- 11.5µm) is the longest among the total chromosome complement and three pairs of chromosomes showing relatively long to short chromosomes (length- 5- 6 µm) with submedian to nearly submedian primary constrictions. The percentage of metacentric chromosome except secondary constriction was 63.6% and the percentage of sub- metacentric chromosome except secondary constriction was 27.3% of this morphotypes. The total centromeric index was 39.86% and the total chromatin length was 68± 0.79 µm. The arm ratio of the total chromosomes of this morphotypes was 1.94. One pair of interstitial secondary constrictions was in this morphotypes. One pair of chromosome (length- 11.5µm), which was longest among the all chromosomes in this morphotypes.

Morphotype-III: The chromosome number of this type was 2n= 22 with the Karyotype formula 2B + 14C + 6D (Table- 3). The ranges of the lengths of chromosomes were 3.5µm – 10µm. One pair of chromosome was relatively long chromosomes (5µm) with primary and secondary constrictions, the median arm is small. The chromosome showing relatively long to short chromosomes with median to nearly median primary constriction of chromosomes (length- 4- 10µm) is the longest among the total chromosome complement and the chromosome showing relatively long to short chromosomes (length- 2- 2.5 µm) with submedian to nearly submedian primary constrictions. The percentage of metacentric chromosome except secondary constriction was 63.6% and the percentage of sub- metacentric chromosome except secondary constriction was 27.3% of this morphotypes. The total centromeric index was 41.60% and the total chromatin length was 54± 0.02 µm. The arm ratio of the total chromosomes of this morphotypes was 1.75. One pair of interstitial secondary constrictions was in this morphotypes. One pair of chromosome (length- 10µm), which was longest among the all chromosomes in this morphotypes.

Morphotype-IV: The chromosome number of this type was 2n= 22 with the Karyotype formula 2A + 16C + 4D (Table- 3). The ranges of the lengths of chromosomes were 4µm - 10µm. One pair of chromosome was relatively long to short chromosomes (4 µm) with primary and secondary constrictions dividing the chromosome into three more or less equal arms. Eight pairs of chromosomes showing relatively long to short chromosomes with median to nearly median primary constriction of chromosomes (length- 4- 10µm) is the longest among the total chromosome complement and two pairs of chromosomes showing relatively long to short chromosomes (length- 4.5- 5 µm) with submedian to nearly submedian primary constrictions. The percentage of metacentric chromosome except secondary constriction was 72.7% and the percentage of sub- metacentric chromosome except secondary constriction was 18.2% of this morphotypes. The total centromeric index was 39.33%µm and the total chromatin length was 54± 0.05 µm. The arm ratio of the total chromosomes of this morphotypes was 1.64. One pair of chromosome (length- 10µm), which was longest among the all chromosomes in this morphotypes.

Morphotype-V: The chromosome number of this type was 2n= 22 with the Karyotype formula 2A + 16C + 4D (Table- 3). The ranges of the lengths of chromosomes were 3.5µm – 11.5µm. One pair of chromosome was relatively long to short chromosomes (6.5 µm) with primary and secondary constrictions dividing the chromosome into three more or less equal arms. Eight pairs of chromosomes showing relatively long to short chromosomes with median to nearly median primary constriction of chromosomes (length- 3.5- 11.5µm) is the longest among the total chromosome complement and two pairs of chromosomes showing relatively long to short chromosomes (length- 5 µm) with submedian to nearly submedian primary constrictions. The percentage of metacentric chromosome except secondary constriction was 72.7% and the percentage of sub- metacentric chromosome except secondary constriction was 18.2% of this morphotypes. The total centromeric index was 40.05%µm and the total chromatin length was 65.5± 0.42 µm. The arm ratio of the total chromosomes of this morphotypes was 1.61. One pair of chromosome (length- 11.5µm), which was longest among the all chromosomes in this morphotypes.

Morphotype-VI: The chromosome number of this type was 2n= 22 with the Karyotype formula 2A + 2B + 12C + 6D (Table- 3). The ranges of the lengths of chromosomes were 4µm – 10.5µm. One pair of chromosome was relatively long to short chromosomes (9.5 µm) with primary and secondary constrictions dividing the chromosome into three more or less equal arms and one pair of chromosome was relatively long chromosomes (7.5µm) with primary and secondary constrictions, the median arm is small. Six pairs of chromosomes showing relatively long to short chromosomes with median to nearly median primary constriction of chromosomes (length- 4- 10.5µm) is the longest among the total chromosome complement and five pairs of chromosomes showing relatively long to short chromosomes (length- 4.5- 5.5 µm) with submedian to nearly submedian primary constrictions. The percentage of metacentric chromosome except secondary constriction was 54.5% and the percentage of sub- metacentric chromosome except secondary constriction was 27.3% of this morphotypes. The total centromeric index was 34.7% and the total chromatin length was 69.5± 0.88 µm. The arm ratio of the total chromosomes of this morphotypes was 2.47. One pair of interstitial secondary constrictions was in this morphotypes. One pair of chromosome (length- 10.5µm), which was longest among the all chromosomes in this morphotypes.

The details comparative karyotypes of the six different morphotypes were of collections are summarized in Table- 3 and Figure- 7.

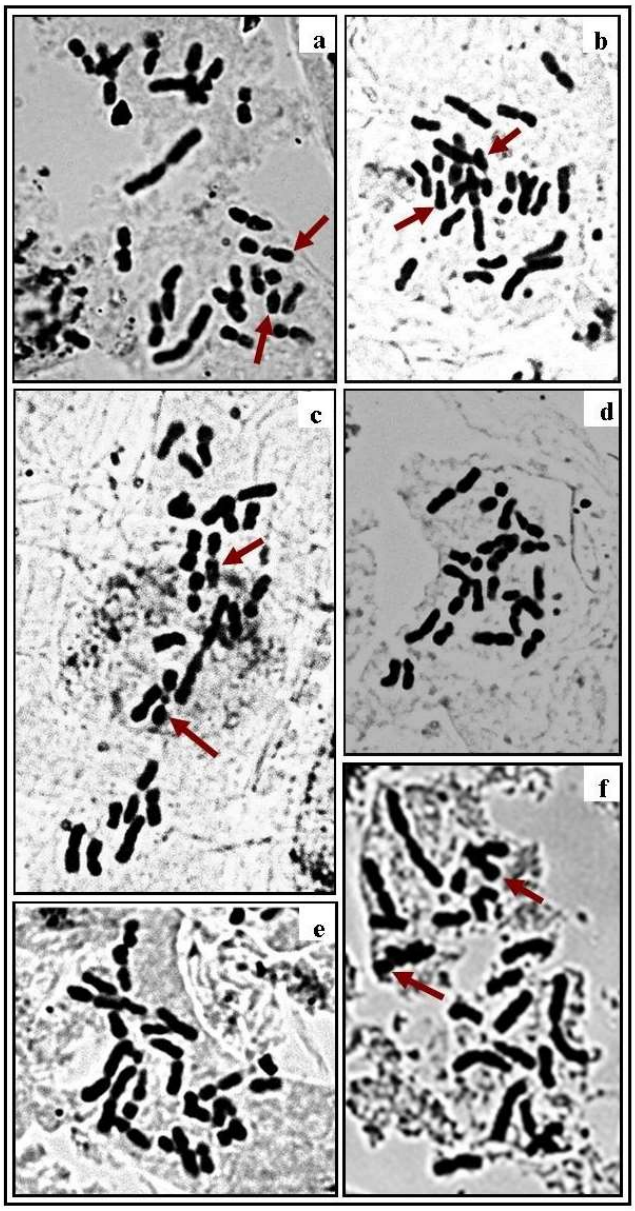


Fig. 4

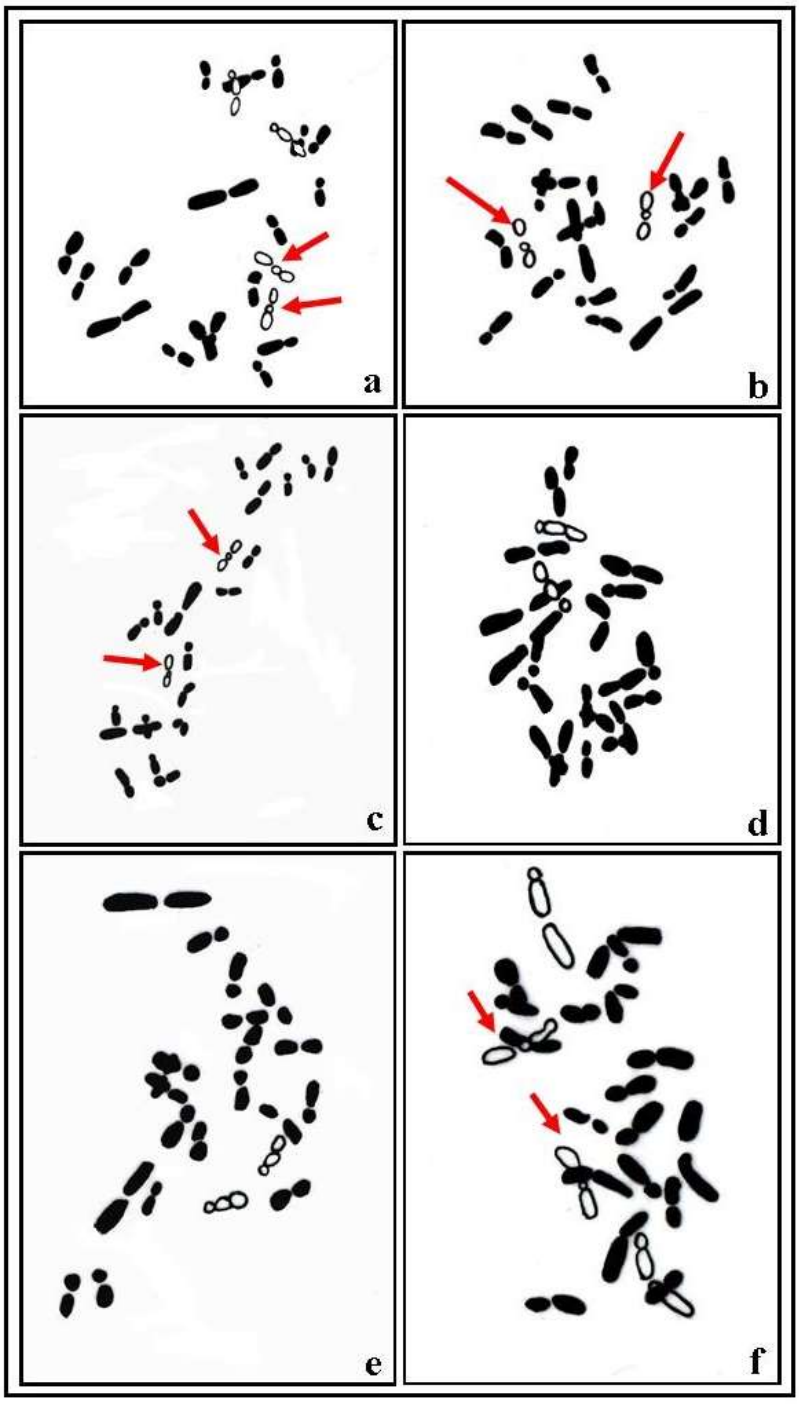


Fig. 5

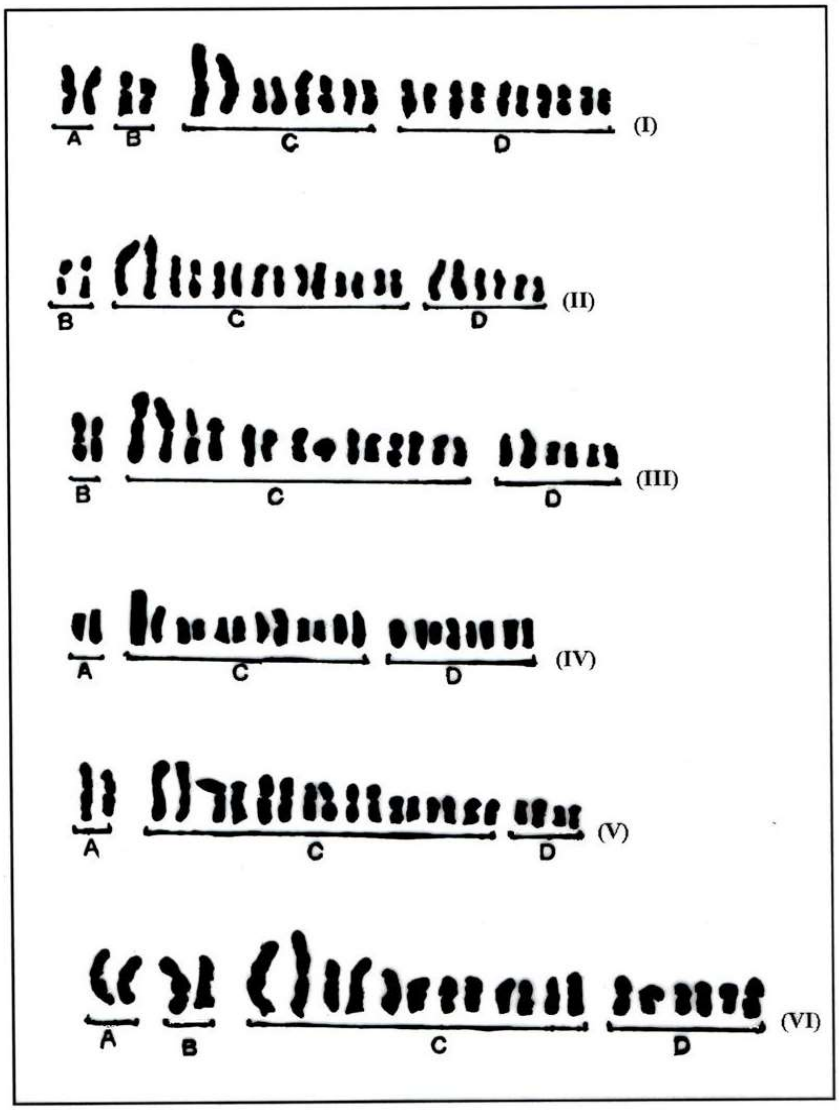


Fig. 6

Fig. 4 (a- f); Showing 2n= 22 metaphase chromosome in different populations of *Gloriosa superba* L. from the state of West Bengal, a- Rahara, b- Narendrapur, c- Sargachi, d- Kalyani, e- Benapur, f- Lava. The arrows are representing the interstitial secondary constrictions.

Fig. 5 (a- f); Camera Lucida drawing shows 2n= 22 metaphase chromosome in different populations of *Gloriosa superba* L. from the state of West Bengal, a- Rahara, b- Narendrapur, c- Sargachi, d- Kalyani, e- Benapur, f- Lava. The arrows are indicating the interstitial secondary constrictions.

Fig. 6 (I– IV); Showing karyotypes of somatic chromosomes in different populations of Gloriosa superba L. from the state of West Bengal, I- Rahara, II- Narendrapur , III- Sargachi, IV- Kalyani, V- Benapur, VI- Lava.

Table-3: Comparative karyotype of different Morphotypes of *Gloriosa superba* L.:

Mor-photypes	Source	No. of 2n chro-mosom e	2n karyotype formula	Total chromatin length (µm)	Length of Long-est chro-mosome (µm)	Length of Smallest chromo-some	L-S (µm)	TF%	% of m	% of Sm	Total arm ratio	Range of length of chromosome (µm)
I	In vivo	22	2A + 2B + 8C + 10D	62.5±0.12	10±0.13	4±0.44	6	38.18	36.4	45.5	2.46	4- 10
II	In vivo	22	2B + 14C + 6D	68±0.79	11.5±0.42	5±0.38	6.5	39.86	63.6	27.3	1.94	5- 11.5
III	In vivo	22	2B + 14C + 6D	54±0.02	10±0.06	3.5±0.41	6.5	41.60	63.6	27.3	1.75	3.5- 10
IV	In vivo	22	2A + 16C + 4D	54±0.05	10±0.03	4±0.05	6	39.33	72.7	18.2	1.64	4- 10
V	In vivo	22	2A + 16C + 4D	65.5±0.42	11.5±0.30	3.5±0.35	8	40.05	72.7	18.2	1.61	3.5-11.5
VI	In vivo	22	2A + 2B + 12C + 6D	69.5+0.88	10.5+0.20	4+0.02	6.5	39.04	54.5	27.3	2.47	4- 10.5

(Morphotypes details: I- Rahara, II- Narendrapur, III- Sargaachi, IV-Kalyani, V- Benapur, VI- Lava)
In columns 5, 6, 7 the results are mean ± Standard Error (calculated from 5 replicates)

Levan, Fredga, and Sandberg (1964) discussed thoroughly the nomenclature of chromosomes, and only salient points of their paper will be taken into consideration in this chapter. The relative lengths of the long arm (l) and short arm (s) are shown by the arm ratio (r= l/s). Based on arm ratio, Levan, Fredga, and Sandberg (1964) grouped chromosomes in six categories (Table-4).

Table- 4: Nomenclature of Chromosomes

Centromere Position	Arm ratio (l/s)	Chromosome Designations
Median sensu stricto	1.0	M (Metacentric)
Median region	1.7	m (Metacwentric)
Submedian	3.0	Sm (Submetacentric)
Subterminal	3.0	St (Subtelocentric)
Terminal	7.0	t (Acrocentric)
Median sensu stricto	-	T (Telocentric)

Levan *et al*., 1964, recommended terms to discard. Because these terms are often used in chromosome nomenclature, I suggest they not abandoned.

Variations in colchicine in different morphotypes

Qualitative presence of colchicine in the tubers of the six collected morphotypes necessitated the quantitative estimation of their colchicine content. Qualitative and quantitative estimation from the tuber extraction from the tuber of *G. superba* L. was done through HPTLC and HPLC techniques (Figure- 6). There were significant variations in colchicine content of all the six morphotypes in West Bengal. The colchicine content in the tubers of *G. superba* L. ranged from 0.13 ± 0.01 to 0.71 ± 0.01. The height concentration of colchicine (0.71%) was observed in the morphotype- VI and the lowest concentration of colchicine (0.13%) was observed in the morphotype- IV. In our study, it was observed that variation of colchicine content depends upon the morphological variations of tubers (as on size and diameter) in different morphotypes. In morphotype- VI, tuber size (12.36 ± 0.2) and diameter of tuber (2.12 ± 0.05) was larger among all the morphotypes and colchicine content was also higher percentage than other morphotypes. But in case of morphotypes-IV, tuber size (7.6 ± 0.1) and diameter of tuber (1.34 ± 0.06) was smallest among all the morphotypes and colchicine content was also lower percentage than other morphotypes. The variations of colchicine content in different morphotypes are summarized in Table-5. Relatively high colchicine content of the morphotype-VI encourages its cultivation in Lava of District of Darjeeling.

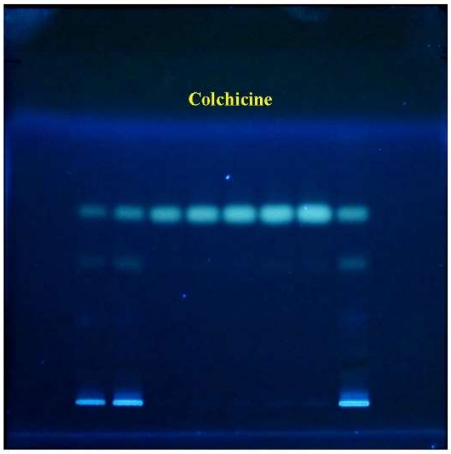


Fig. 7. HPTLC analysis of Colchicine content from the tuber extract of *Gloriosa superba.*

4. Discussion

Gloriosa superba L. of liliaceae, which in later classification system transferred to colchicaceae is most important plant for its medicinal value in general and as the source of colchicines in particular (Amano *et al*., 2008). Detail karyomorphological analysis and content of colchicine analysis of six different morphotypes of West Bengal were carried out first time in our laboratory. Somatic chromosome number of all six morphotypes of *G.superba* L. showed 2n= 22 (Figure- 4, 5). At the same time the TF% and total arm ratio of all the morphotype suggest the symmetrical karyotype of this species. These findings are similar to the earlier reports (Sharma and Sharma, 1980; Vijayavalli and Mathew, 1990 and Biswas *et al*., 2014). Chromosomal polymorphism of this species was noted as 2n= 56 (Nowfia *et al*, 2001) and 2n= 44 (Ghosh *et al*., 2008). In our study no chromosomal polymorphism was observed in any morphotypes. However critical observation revealed that there are variations of karyotypes in terms of karyotype formula the numbers of sub- metacentric chromosomes and types of secondary constrictions, total TF% and total arm ratio, percentage of metacentric and sub metacentric chromosome. In morphotype-VI, TLC is 69.5 µm which is highest among the all whereas in morphotype- III and IV TCC is lowest i.e. 54 µm. Presence of interstitial secondary constrictions

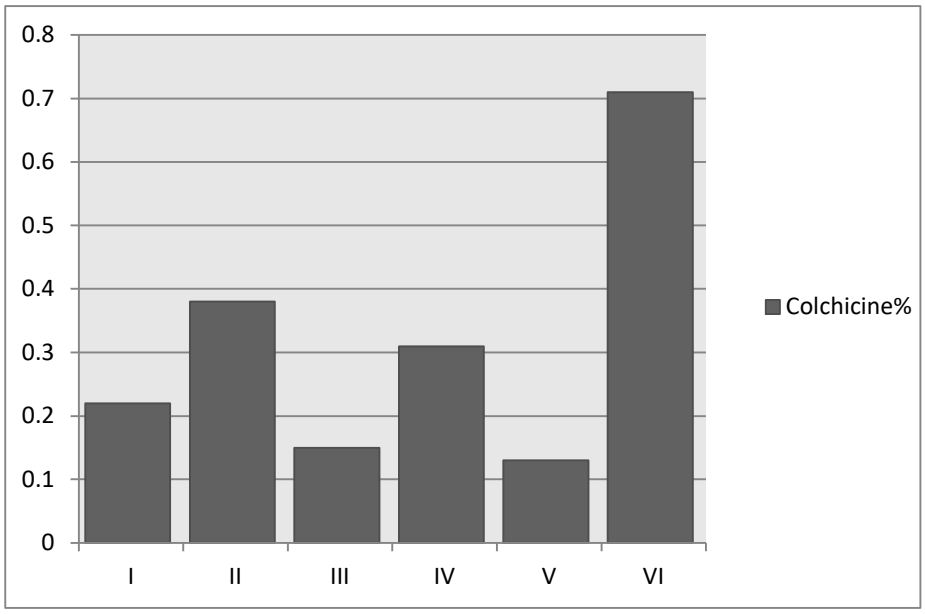
in the morphotype-I, morphotype-II, morphotype-III and morphotype-VI, are first time reported in this species. At the same time a pair of longest chromosome with median primary constrictions in chromosome complement of all the morphotypes may be consider as the characteristics of this species. However, the presence of a heteromorphic metacentric chromosome pair (10 µm + 9 µm) in morphotype- IV (Kalyani) supports the earlier report by Vijayavalli and Mathew, 1990. Consequently, a combination of features such as mean chromatin length, number and types of secondary constricted chromosomes and karyotype formula allow us to distinguish the karyotypes of specific morphotypes of *G. superba* of West Bengal. Similarly, the variations of karyotype features among the morphotypes indicate the minor structural changes of chromosomes and gene mutation which may be cause of the intraspecific evolutions.

The variation of colchicine content (Table no.-5), may therefore suggest that there is no corelations between the variations of karyotypes of the six morphotypes. The high colchicine content (0.71%) in the morphotype of Lava in which TLC is highest (69.5 µm) may be due to the over expression of specific genes in this temperate climatic conditions. The TLC value (69.5 µm) of Lava morphotype is not significantly high among the all (Table-4). Evidently, variation of colchicine content in the morphotypes is may depend on the climatic condition.

Table-5: Colchicine content of tubers (1 yr. old) of the plants of the six morphotypes collected. Values represent means ± standard error of three experiments with 10 replicates.

Morphotypes	Colchicine content (% d.wt)
Morphotype- I	0.22 ± 0.02
Morphotype- II	0.38 ± 0.0
Morphotype- III	0.15 ± 0.01
Morphotype- IV	0.13 ± 0.01
Morphotype- V	0.31 ± 0.02
Morphotype- VI	0.71 ± 0.01

Relatively high colchicine content of the morphotype-VI encourages its cultivation in Lava of District of Darjeeling.



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6. References

Ade, R., Rai, K. M. 2009. Review: Current Advances in *Gloriosa superba* L. Biodiversitas. 10: 210- 214.

Amano, J., Kuwayama, S., Mizuta, Y, Nakano, M. 2008. Morphological Characterization of Three Intergeneric Hybrids Among *Gloriosa superba*'Lutea', *Littoniamodesta*, and *Sandersoniaaurantica* (Colchicaceae). Hort Science. 43: 115-118.

Biswas, A., Muntaha, S.N., Rahman, M.M. 2014. Comparative karyotype analysis in two life-forms of *Gloriosa superba* L.Journal of Pharmaceutical Biology. 4: 77-80.

Fedorov, A. 1969. Chromosome Numbers of Flowering Plants, (ed.) V. L. Komarov. Botanical Institute, Leningard.

Ghosh, S., Ghosh, B., Jha, S. 2008.Polymorphism in *Gloriosa superba*. Plant Genetic Resource: Characterization and Utilization. 1- 7.

Ghosh, B., Mukherjee, S., Jha, B.T., Jha, S. 2002. Enhanced colchicine production in root cultures of *Gloriosa superba*by direct and indirect precursors of the biosynthetic pathway.Biotechnology Letters.24: 231- 234.

Goldblatt, P. 1984. Index to Plant Chromosome Number 1979- 81. Missouri Botanical Garden Vol. 8. Braun, Brumfield, Inc., Ann Arbor, Michigan.

Gupta, L. M., Raina, R. 2001. Significance of sequential opening of flowers in *G. superba* L. Curr Sci. 80: 1266-7.

Huziwara, Y. 1962. Karyotype analysis in some genera of Compositae VIII. Further Studies on the chromosomes of Aster. American Journal of Botany. 49: 116-119.

Hayashi, T., Yoshida K., Sano K., 1988. Formation of alkaloids in suspension cultured *Colchicum autumnale*. Phytochemistry. 27: 371- 1374.

Jana, S., Shekhawat, S. G. 2011. Critical review on medicinally potent plant species: *Gloriosa superba*. Fitoterapia. 82: 293- 301.

Kavina, J., Gopi, R., Panneerselvam, R. 2011. *Gloriosa superba* Linn- A Medicinally important plant. Drug Invention Today. 3: 69-71.

Levan, A., Fregda, K., Sandberg, A.A. 1964. Nomenclature on centromeric position on chromosomes. Heredities. 52: 201-220.

Maroyi, A., Maesen, D.V.G.J.L. 2011. Gloriosa superba L. (family Colchicaceae): Remedy or poison? Journal of Medicinal Plant Research. 5: 6112- 6121.

Nowfia, E.G.; Madu, A.O., Ene- Obong, E.E. 2001. Studies on the chromosomes of tropical lilies 2: karyotype of Gloriosa superba lindle (liliaceae). Journal of Applied Chemistry and Agricultural Research. 7: 54- 57.

Rajagopal, C., Kandhasamy, R. 2009. Genetic variability of kazhappaikizhangu (*Gloriosa superba*) in Tamil Nadu assessed using morphological and biochemical traits. Journal of Tropical Agriculture. 47: 77- 79.

Sharma, A.K., Sharma, A. 1980. Chromosome techniques- theory and practice. 3rd ed. London: Butterworths.

Vijayavalli, B., Mathew, M.P. 1990. Karyomorphology of four Morphotypes of *Gloriosa superba* L. from South India.Cytologia. 55: 531-533.